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A SYSTEMATIC REVIEW OF THE CORRELATION BETWEEN DYSLEXIA AND THE  
AXON GUIDANCE RECEPTOR GENE, *ROBO1*

By Catherine Day

A thesis submitted to the faculty of The University of Mississippi in partial fulfillment of the requirements of the Sally McDonnell Barksdale Honors College.

Oxford, MS  
April 2021

Approved by

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Advisor: Dr. Tossi Ikuta

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Reader: Dr. Gregory Snyder

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Reader: Dr. Peter Grandjean

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Catherine Day

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I would like to dedicate this research to my brother who through his journey as a pediatric stroke survivor has set an example of resilience and perseverance in overcoming what some would say are insurmountable obstacles to achieve his life goals. Timmy, you are such an inspiration.

## ACKNOWLEDGMENTS

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I would also like to thank all of my professors at the University of Mississippi for preparing me for serving those with speech and language disabilities. I am so grateful for all of my classroom experiences that have prepared me for success in this field.

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## ABSTRACT

CATHERINE DAY: A Systematic Review of the Correlation Between Dyslexia and the Axon Guidance Receptor Gene, *ROBO1*  
(under the direction of Dr. Tossi Ikuta)

This thesis was conducted to investigate further the correlation between the ROBO1 gene and Developmental Dyslexia (DD). DD is when a person is deficient in acquiring literary proficiency even though there is not an intellectual deficit or lack of opportunity to obtain literary skills in an educational environment. Persons with DD are characterized as having difficulties with word recognition, reading fluency, poor spelling, and basic decoding skills. There are several genes that have been evaluated in previous studies as indicators of DD: KIAA0319, DCDC2, DIP2A, and ROBO1. This study is focused on identifying previous published studies that examined specifically the correlation of the ROBO1 gene and DD to summarize those findings to determine if there is a cumulative correlation of findings supporting the ROBO1 gene as an indicator for DD. The results of this study can be used to further target specific ROBO1 SNP markers that through the cumulative data presented in this study have indicated a pattern of correlation for DD.

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## **List of Abbreviations**

DD	Developmental Dyslexia
ESL	English as a Second Language
RD	Reading Disability
ROBO1	Roundabout Guidance Receptor 1
SNPs	Single Nucleotide Polymorphisms



## Introduction

Reading is an ability that is unique to the human race. There are many contributing factors that impact the development of reading skills and the level of competency of one's reading ability. "It is well known that a substantial amount of the variance in reading ability is explained by inherited factors: genetic variance explains about 20–80% of the total variation in reading skills" (Carrion Castillo et al., 2017). However, there is still little known about the genetic predisposition for persons with DD. "The genetic variants that have been identified so far only explain a tiny fraction of estimated heritability" (Carrion Castillo et al., 2017). So where environment and other factors may also contribute to DD, there is a compelling body of evidence that DD runs in families and seems to be highly inheritable. "Several investigations during the last two decades have shown possible locations of genes that might be involved in dyslexia, including regions of chromosomes 1, 2, 3, 6, 11, 13, 15 and 18. In addition, six candidate genes (KIAA0319, DYX1C1, DCDC2, ROBO1, MRPL19 and C2ORF3) seem to be related to dyslexia" (Svensson et al., 2011).

"The ability to read depends on phonological awareness, which is the ability to reflect on the sound structure of words, and the ability to phonologically decode, which is the ability to match phonetic pieces to their written equivalents" (Carrion Castillo et al., 2017). Learning to read requires the accumulation of many cognitive processes, and if any one of those cognitive processes are impaired, the ability to develop reading skills will be impacted. Being able to identify genetic markers that provide early identification would open the doors to provide early intervention therapies to children who show a predisposition for DD. Genetic research has found that "strong genetic correlations between language, mathematical and reading traits have been consistently reported" (Mascheretti et al., 2014). Early identification is important because it has

been found that through early identification which leads to early intervention promotes a long-term positive prognosis for those struggling with DD. By investigating and identifying genetic markers that determine a child may be at risk for DD, this research will open doors for early identification and intervention for many struggling with DD.

## **Literary Research of Dyslexia**

### ***Developmental Dyslexia (DD) Introduction***

“Developmental Dyslexia (DD) is a neurodevelopmental disorder that is the most common childhood learning disorder and a significantly inheritable trait.” (Svidnicki et al., 2013). Dyslexia can be diagnosed in the first years of school, and about 5-17% of school age children are diagnosed with some level of dyslexia. [Beitchman et al., 1996; Lyon et al., 2003]. “Dyslexia is a neurodevelopmental disorder that manifests as a reading disability despite normal intelligence and adequate educational opportunity. Twin and family studies have indicated a genetic component, while genome-wide studies have implicated a number of susceptibility genes, most of which have direct or indirect roles in neuronal migration,” (Devasenapathy et al., 2018) “When diagnosed with dyslexia, children can begin a downward spiral of low self-esteem, underachievement, poor mental health, isolation and social disadvantages” (Svidnicki et al., 2013).

### ***Dyslexia Symptoms***

Because there are many symptoms associated with dyslexia, this makes for a large population to evaluate for diagnosis. While dyslexia impacts the ability to read, dyslexia is not an indicator of intelligence. Dyslexia is a specific impairment related to reading that is indicated by tests showing a substantially low reading ability when compared to the person’s chronological

age (Venkatesh et al., 2013). A deficiency in spelling and writing skills are usually affected, but the overall cognitive ability of the diagnosed is not affected (Kere, 2011). Individuals that have been diagnosed with dyslexia have been shown to perform lower on non-word repetition assignments (Tran et al., 2014). The central features that go along with dyslexia include phonological deficits, impaired reading fluency, short-term memory problems and difficulties with rapid naming (Devasenapathy et al., 2018). The speed and the accuracy of word decoding are two more symptoms that have been found with the dyslexic population along with text comprehension (Svidnicki et al., 2013). Word recognition is also affected leading to a slow and inaccurate word recognition ability even though intelligence and sensory abilities are normal (Kong et al., 2016). In addition to reading skills, an essential discrepancy is found when processing phonemes, which are the basic units of speech sounds (Tran et al., 2014).

### **Literary Research of the ROBO1 Gene**

#### ***ROBO1 Gene Introduction***

ROBO1 is well known as the guidance receptor gene that is a possible influencer for reading performance and dyslexia as it is the gene that plays a crucial role in the Central Nervous System as the axon growth across the midline (Sun et al., 2017). It is noted that the ROBO1 gene encodes an integral membrane protein that is both an axon guidance receptor and a cell adhesion receptor (Venkatesh et al., 2013). “Biologically, the ROBO1 gene encodes a receptor that acts as molecular guidance cue during cellular migration and axonal navigation. Specifically, this receptor has been found to play a critical role in axon growth across the midline of the brain.” (Sun et al., 2017; Kidd et al., 1998; Lei et al., 2011; Wong et al., 2002). In addition, there has been works that have shown that an SLI-related phenotype, speech sound disorder, has shown linkage to the region on chromosome 3 in which ROBO1 is located. Thus, revealing the roles of

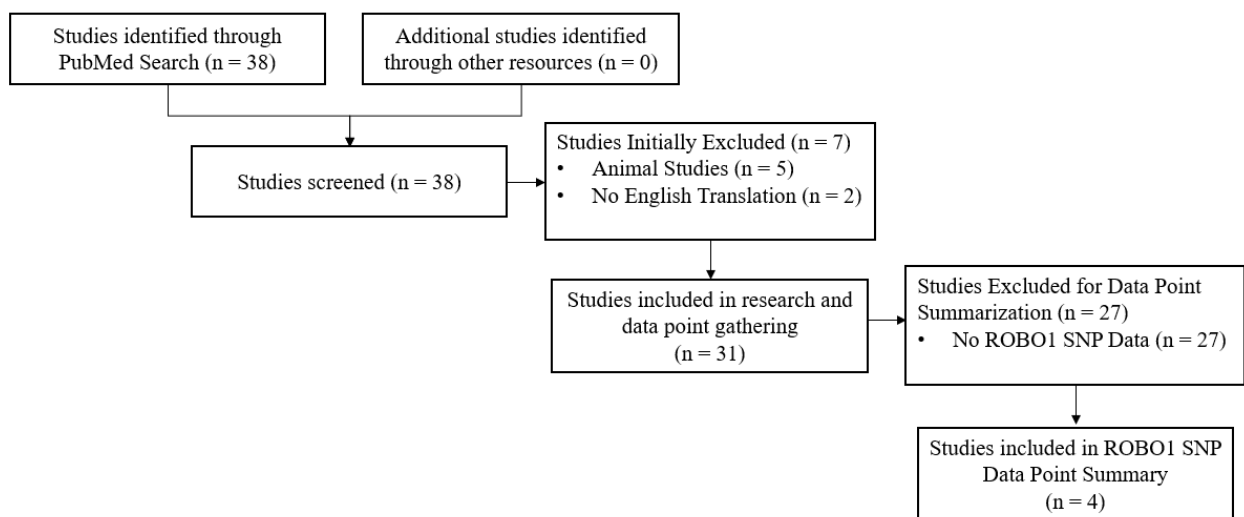
ROBO1 in human neurodevelopment seems highly relevant (Lamminmäki et al., 2012). In studies, ROBO1 in addition to other susceptibility genes in the chromosome, have shown correlation as a significant influencer on reading scores (Sun et al., 2017).

## Methods

### Data Acquisition

To determine eligible sources for this summarization of published research studies, a search of PubMed was done identifying 38 published studies correlating the ROBO1 gene and dyslexia. Non-human studies and studies without an English translation were then excluded.

Figure 1. Flow Diagram of Eligible Studies

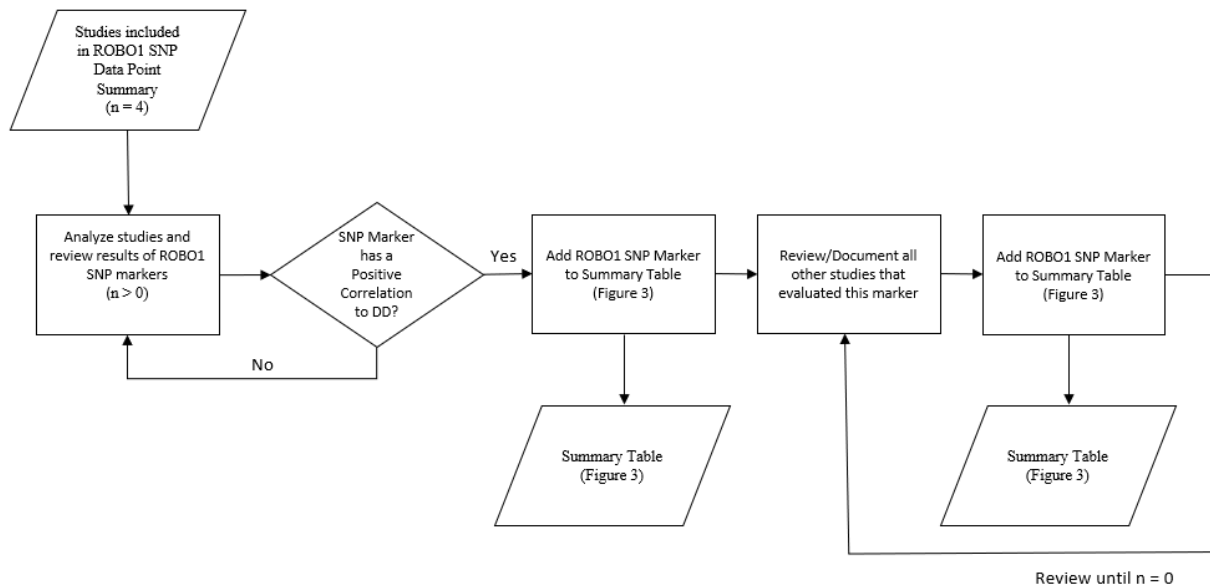


Of the original 38 PubMed studies correlating the ROBO1 gene and dyslexia, 31 studies met the criteria for inclusion of relevant human research of ROBO1 and DD. Studies that were eliminated included 5 that were non-human studies and 2 that were eliminated because there was no English translation. After evaluating each of the 31 remaining studies, only 4 of the 31 published studies included data relevant for summarizing ROBO1 specific SNP markers. See Figure 1 for the inclusion and exclusion diagram.

## Data Summarization

While there were several studies that spoke to the theoretical relationship of the ROBO1 gene and DD as it related to prior studies, there were only 4 studies found that performed a trial of sample participants with known DD that provided data supporting the evaluation of the correlation with the ROBO1 gene and identification of the corresponding SNP marker. There were four studies that investigated 1 or more ROBO1 SNPs correlation to a sample population of DD.

Figure 2. Flow Diagram of Data Summarization Process



Of these four studies, if there was an SNP marker evaluated and it showed a positive correlation with DD, it was included in the summary data in Figure 2. If one of the other studies had the same SNP marker that was evaluated, it was also included in the summary whether the correlation was negative or positive. The intent of this summarization method was to determine if across these studies if there was an SNP marker that was consistently showing a correlation.

The study, ROBO1 Polymorphisms, Callosal Connectivity, and Reading Skills, was included in

this analysis because it specifically studied the two SNP's, ROBO1 rs4535189/rs6803202. This study revealed a "ROBO1-callosum-reading pathway" tying the ROBO1 rs4535189/rs6803202 to word list and reading performance by modulating the fiber microstructures of the genu of the CC (Sun et al., 2017). This study laid the groundwork for demonstrating direct evidence of a ROBO1-callosum association as well as provided insight into the connection of the gene-to-brain mechanisms that impact human reading. These two SNP's, rs4535189/rs6803202, are included in the results summary for this study to assimilate findings from other studies that also evaluated rs4535189/rs6803202 as possible indicators of DD.

## Results

Of the ROBO1 SNP Markers identified in the four selected published studies, only three had SNP Markers showing a positive correlation to DD. Across these three studies, five SNP markers were identified as having at least one positive finding: rs4535189, rs6803202, rs331142, rs12495133, and rs1995402. Three of the markers had inconsistent findings (indicated in one study but not in another): rs4535189, rs6803202, rs331142. One marker, rs1995402, had no negative findings, but it was only included in one study. The only marker that was indicated across more than one study with no negative findings in other studies was rs12495133.

**Table 1:** SNP Summary of Findings Across Published Studies

ROBO1 - SNP Marker Evaluated	Reference	Sample Size Families/Cases	Reported Best P Value	Study Population Origin	Indicated
rs4535189	(3)	115 Children	N/A **	China	<b>Yes</b>
	(14)	151 Families (Toronto)	0.797	Canada	No
	(27)	538 Families	9.30 x 10-05	Australia	<b>Yes</b>
rs6803202	(3)	115 Children	N/A **	China	<b>Yes</b>

	(14)	151 Families (Toronto)	0.794	Canada	No
	(27)	538 Families	8.70 x 10-05	Australia	<b>Yes</b>
rs331142	(14)	131 Families (Toronto)	0.001	Canada	<b>Yes</b>
	(14)	43 Families (Calgary)	0.196	Canada	No
rs12495133	(14)	158 Families (Toronto)	0.005	Canada	<b>Yes</b>
	(14)	61 Families (Calgary)	0.007	Canada	<b>Yes</b>
rs1995402	(27)	538 Families	0.04	Australia	<b>Yes</b>

\*\* This study evaluated MRI brain scan findings with participants with wordlist and reading performance, an indicator of DD. See Figure 3 for more details on this study.

In the evaluation process of the published studies, one study, A Pilot Indian family-based Association Study Between Dyslexia and Reelin Pathway Genes, DCDC2 and ROBO1, identifies modest association with a triallelic unit TAT in the gene RELN (Devasenapathy et al., 2018), set out to show a correlation between ROBO1 and DD, but all of the SNP markers evaluated did not show an indicated correlation (*see Table 2 below*). The results from this study were then not included in **Table 1: SNP Summary of Findings Across Published Studies** because there was no positive correlation to DD for these ROBO1 markers that were evaluated.

**Table 2:** ROBO1 SNP Marker findings from the published study: A pilot Indian family-based association study between dyslexia and Reelin pathway genes, DCDC2 and ROBO1, identifies modest association with a triallelic unit TAT in the gene RELN (Devasenapathy et al., 2018)

ROBO1 - SNP Marker Evaluated	Reference	Sample Size Families/Cases	Reported Best P Value	Study Population Origin	Indicated
rs723766	(1)	102 Children/Adults	0.78	India	No
rs6795556	(1)	102 Children/Adults	0.30	India	No
rs3773195	(1)	102 Children/Adults	0.75	India	No



The published study, “Association of the ROBO1 gene with reading disabilities in a family-based analysis” (Tran et al., 2014) evaluated 16 SNPs in two studies, one done in Toronto, Canada evaluating 16 SNPs and another in Calgary, Canada evaluating 34 SNPs. The SNPs in these studies with a positive indication for DD were included in **Table 1: SNP Summary of Findings Across Published Studies** as well as any SNP marker that had an indicated correlation in one of the other evaluated studies.

## **Discussion**

In this study, we aimed to collect published studies evaluating ROBO1 SNP markers to strengthen the theory of the correlation of the ROBO1 gene and DD. Of the 38 published studies evaluated, only four had data points for human trials evaluating the correlation of the ROBO1 gene and dyslexia. Performing a metadata analysis on existing studies that evaluated the correlation between SNP markers would be an insightful way to assimilate the findings of existing published research to better understand the depth of the theorized correlation of the ROBO1 gene and DD. For a metadata analysis to be statistically relevant, more studies providing additional data points are needed than what are currently available in existing published studies.

This study highlights ROBO1 SNP markers that have been evaluated across published studies to understand the correlation of findings. In this study, it is shown that of the published studies reviewed, there is only one SNP marker, rs12495133, that was indicated as having a positive correlation in more than one study and no negative indication in any of the other evaluated studies.

This study also provides further insight to the published study in China that laid the groundwork for demonstrating direct evidence of a ROBO1-callosum association and gene-to-

brain mechanisms that impact human reading that found this correlation with the ROBO1 rs4535189/rs6803202 markers. Our study found that these markers, rs4535189/rs6803202, were also part of the study done in Toronto, Canada. As opposed to the study in China, the study in Toronto, did not find rs4535189/rs6803202 markers to be an indicator of DD in that studies' participants.

In summary, the results of this study are inconclusive. To show a definitive correlation of the ROBO1 gene to DD, more trials need to be performed across a larger sample size of participants before a meaningful summary of results or metadata analysis can be performed. Also, the published studies that provided SNP data that could be included in this study were from the countries of origin of Australia, China, and Canada. Further studies in other countries, including the United States, with a defined constant metric of identification of participants with DD (i.e WISC, Woodcock Reading Mastery Test, Revised Woodcock–Johnson Psychoeducation Test, etc.) would provide needed additional data points to evaluate the ROBO1 genetic correlation to DD.

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